Our Ref. No.: 36119.140US3

Reply to Office Action of Nov. 21, 2005

**Amendments to the Claims:** 

This listing of claims will replace all prior versions, and listings, of claims in the instant

application:

**Listing of Claims:** 

Claims 1-58. (Canceled)

Claim 59. (New): A method for increasing the expression of an exogenous nucleic acid

molecule in T cells, comprising:

(a) contacting the T cells in vitro with at least one stimulatory agent, wherein the T

cells are proliferating prior to contact with the at least one stimulatory agent, thereby

forming stimulated proliferating T cells; and

(b) introducing the exogenous nucleic acid molecule into the T cells from step (a) in vitro,

less than 24 hours after contacting of said T cells, wherein the exogenous nucleic acid

molecule is introduced into the T cells using a viral vector, provided that the exogenous

nucleic acid molecule is not introduced by particle bombardment,

such that the expression of the exogenous nucleic acid molecule is increased in the T cells

compared with T cells not contacted with the stimulatory agent prior to introducing the

exogenous nucleic acid molecule.

Claim 60. (New): The method of claim 59, wherein the T cells are contacted *in vitro* with at

least one proliferative agent which stimulates proliferation of the T cells prior to being contacted

with the at least one stimulatory agent.

Claim 61. (New):

The method of claim 59, wherein the T cells are primary T cells.

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Claim 62. (New): The method of claim 59, wherein the at least one stimulatory agent is a

combination of a phorbol ester and a calcium ionophore, a super-antigen, a polyclonal activator,

a lymphokine, an antigen presented by an antigen presenting cell, or a protein tyrosine kinase

activator.

Claim 63. (New):

The method of claim 59, wherein the at least one stimulatory agent is an

antibody.

Claim 64. (New): The method of claim 59, wherein the at least one stimulatory agent is an

agent which interacts with the T cell receptor/CD3 complex and provides a primary activation

signal to the proliferating T cells.

Claim 65. (New): The method of claim 64, wherein the agent which interacts with the T cell

receptor/CD3 complex is an agent which interacts with the T cell receptor, an agent which

interacts with the CD3 complex, or an agent that stimulates the CD2 complex on T cells.

Claim 66. (New): The method of claim 59, wherein the stimulatory agent is an anti-CD3

antibody, or a combination of anti-CD2 antibodies.

Claim 67. (New):

The method of claim 59, wherein the stimulatory agent is attached to a

surface.

Claim 68. (New):

The method of claim 67, wherein the surface is a bead, a cell surface, or a

tissue culture dish.

Claim 69. (New): The method of claim 59, wherein the at least one stimulatory agent is a

combination of a first agent which provides a primary activation signal to the proliferating T

cells, and a second agent which provides a costimulatory signal to the proliferating T cells.

Claim 70. (New): The method of claim 69, wherein the first agent is an agent which interacts with the T cell receptor/CD3 complex and provides a primary activation signal to the proliferating T cells.

Claim 71. (New): The method of claim 69, wherein the first agent is an anti-CD3 antibody.

Claim 72. (New): The method of claim 69, wherein the first agent interacts with a CD2 complex on the T cells.

Claim 73. (New): The method of claim 69, wherein the first agent is an antigen on an antigen presenting cell.

Claim 74. (New): The method of claim 69, wherein the second agent is an anti-CD28 antibody.

Claim 75. (New): The method of claim 69, wherein the second agent is a stimulatory form of a natural ligand of CD28.

Claim 76. (New): The method of claim 75, wherein the stimulatory form of a natural ligand of CD28 is the B lymphocyte antigen B7-1.

Claim 77. (New): The method of claim 75, wherein the stimulatory form of a natural ligand of CD28 is the B lymphocyte antigen B7-2.

Claim 78. (New): The method of claim 69, wherein the first agent or the second agent is attached to a surface.

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Claim 79. (New): The method of claim 69, wherein the first agent and the second agent are

attached to a surface.

Claim 80. (New): The method of claim 79, wherein the first agent and the second agent are

attached to the same surface.

Claim 81. (New): The method of claim 78, wherein the surface is a bead, a cell surface, or a

tissue culture dish.

Claim 82. (New): The method of claim 59, wherein the viral vector is selected from the

group consisting of recombinant retroviruses, adenovirus, adeno-associated virus, and herpes

simplex virus-1.

Claim 83. (New):

The method of claim 59, wherein the viral vector is a recombinant

retrovirus.

Claim 84. (New):

The method of claim 59, wherein the recombinant retrovirus is replication

defective.

Claim 85. (New): The method of claim 59, wherein said nucleic acid molecule is introduced

into said T cells, between approximately 1 hour and less than 24 hours after contacting said T

cells in vitro with said at least one stimulatory agent.

Claim 86. (New): The method of claim 59, wherein said nucleic acid molecule is introduced

into said T cells, approximately 10 hours after contacting said T cells in vitro with said at least

one stimulatory agent.

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Claim 87. (New): The method of claim 59, wherein the T cells of step (b) are further stimulated *in vitro* to increase their number.

Claim 88. (New): The method of claim 59, wherein the T cells are obtained from a subject, and are readministered to the subject after introducing the exogenous nucleic acid molecule into the T cells.

Claim 89. (New): A method for increasing the expression of an exogenous nucleic acid molecule in T cells, comprising:

- (a) contacting the T cells with at least one proliferative agent which stimulates proliferation of the T cells, forming proliferating T cells;
- (b) contacting the proliferating T cells *in vitro* with at least one stimulating agent, thereby forming stimulated proliferating T cells, wherein the at least one stimulatory agent is a combination of a first agent which provides a primary activation signal to the T cells and a second agent which provides a costimulatory signal to the T cells; and
- (c) introducing the exogenous nucleic acid molecule into the T cells from step (b) *in vitro*, less than 24 hours after contacting of said T cells, wherein the exogenous nucleic acid molecule is introduced into the T cells using a viral vector, provided that the exogenous nucleic acid molecule is not introduced by particle bombardment,

such that the expression of the gene is increased in the T cells compared with T cells not contacted with the stimulatory agent prior to introducing the exogenous nucleic acid molecule.

Claim 90. (Previously Presented): The method of claim 89, wherein the T cells are primary T cells.

Claim 91. (New): The method of claim 89, wherein the first agent is an agent which interacts with the T cell receptor/CD3 complex and provides a primary activation signal to the proliferating T cells.

Claim 92. (New): The method of claim 91, wherein the first agent is an anti-CD3 antibody.

Claim 93. (New): The method of claim 91, wherein the first agent interacts with a CD2 complex on the T cells.

Claim 94. (New): The method of claim 91, wherein the first agent is an antigen on an antigen presenting cell.

Claim 95. (New): The method of-claim 89, wherein the second agent is an anti-CD28 antibody.

Claim 96. (New): The method of claim 89, wherein the second agent is a stimulatory form of a natural ligand of CD28.

Claim 97. (New): The method of claim 96, wherein the stimulatory form of a natural ligand of CD28 is the B lymphocyte antigen B7-1 or B7-2.

Claim 98. (New): The method of claim 89, wherein the first agent or second agent is an antibody.

Claim 99. (New): The method of claim 89, wherein the first agent and the second agent are antibodies.

Claim 100. (New): The method of claim 89, wherein the first agent or the second agent is attached to a surface.

Claim 101. (New): The method of claim 89, wherein the first agent and the second agent are attached to a surface.

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Claim 102. (New): The method of claim 101, wherein the surface is a bead, a cell surface, or a

tissue culture dish.

Claim 103. (New): The method of claim 89, wherein the viral vector is selected from the

group consisting of recombinant retroviruses, adenovirus, adeno-associated virus, and herpes

simplex virus-1.

Claim 104. (New):

The method of claim 89, wherein the viral vector is a recombinant

retrovirus.

Claim 105. (New):

The method of claim 89, wherein the recombinant retrovirus is replication

defective.

Claim 106. (New): The method of claim 89, wherein said nucleic acid molecule is introduced

into said T cells, between approximately 1 hour and less than 24 hours after contacting said

proliferating T cells in vitro with said at least one stimulatory agent.

Claim 107. (New): The method of claim 89, wherein said nucleic acid molecule is introduced

into said T cells, approximately 10 hours after contacting said proliferating T cells in vitro with

said at least one stimulatory agent.

Claim 108. (New): The method of claim 89, wherein the T cells of step (c) are further

stimulated in vitro to increase their number.

Claim 109. (New): The method of claim 89, wherein the T cells are obtained from a subject

and are readministered to the subject after introducing the exogenous nucleic acid molecule into

the T cells.